Respectfully submitted,

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In the Specification:

The paragraph starting on page 2, line 17 has been amended as follows:

Heparin-binding consensus sequences were discovered by Cardin and Weintraub, who surveyed amino acid sequences of known heparin-binding proteins, where they identified two potential consensus sequence motifs for heparin-binding, X-B-B-X-B-X or X-B-B-B-X-X-B-X, where X represents a hydropathic or uncharged amino acid, and B a basic amino acid. (Cardin, A. D. and H. J. R. Weintraub, *Arteriosclerosis* **9**:21-32, 1989). For example, such consensus sequences were identified in proteins including apolipoprotein B-100, apo E, and vitronectin, to name a few. (See Cardin and Weintraub, 1989, for review). Molecular modeling of these consensus sites predicts the arrangement of amino acids into either α-helices or β-strands. This allows for the clustering of noncontiguous basic amino acids on one side of the helix, thus forming a charged domain to which GAGs could bind. Indeed, for some heparin-binding proteins, disruption of the heparin-binding consensus sequences hinders heparin binding. For example, chemical modification of the heparin-binding consensus site in thrombospondin (Lawler, J. and R. O. Hynes, *Cell Biol* **103**:1635-1648, 1986) or site-directed mutagenesis of a heparin-binding sequence in fibronectin (FN) (Barkalow, F. J. B. and J. E. Schwarzbauer, *J Biol Chem* **266**:7812-7818, 1991) eliminates or diminishes heparin-binding affinity. On the other hand, peptide mimetics of proposed heparin binding consensus sequences often fail to reveal the high affinities demonstrated by the native heparin-binding proteins. (Conrad, H. E, *Heparin-Binding Proteins*. Academic Press, 1998). Proteins often contain multiple, distal heparin-binding sequences that may come into proximity upon protein folding or multimerization, hence enabling binding through cooperativity. It has thus been speculated that the three dimensional arrangement of multiple heparin-binding consensus sites within or between heparin-binding proteins, and/or the presence of novel heparin-binding sites may be responsible for high affinity heparin- or HS-interactions with native proteins. Others have proposed a necessary approximately 20 Å distance between basic amino acids for heparin binding, regardless of protein tertiary structure. (Margalit, H., et

al., *J Biol Chem* **268**:19228-19231, 1993). Other heparin-binding sequences have been proposed that are variations of those reported by Cardin and Weintraub. The sequence TXXBXXTBXXXTBB, where T is a turn, was identified as a heparin-binding sequence in acidic FGF and bFGF. (Hileman, R. E., et al., *BioEssays* **20**:156-167, 1998). X-ray crystallography revealed that this peptide backbone loops back upon itself in three turns to form a positively charged triangular heparin-binding pocket. The heparin-binding domain of von Willebrand factor resembles the motif XBBXXBBBXXBBX, a palindromic sequence in which the spacing and clustering of basic residues is important for heparin binding. (Sobel, M., et al., *J Biol Chem* **267**:8857-8862, 1992). A third novel sequence has been demonstrated to be sufficient for weak heparin-binding in thrombospondin: WSXW. (Guo, N. H., et al., *J Biol Chem*, **267**:19349-19355, 1992). However, for high affinity binding, this sequence must be flanked by basic residues. Other proteins including type I collagen (Sweeney, S. M., et al., *PNAS* **95**:7275-7280, 1998), type VI collagen (Specks, U., et al., *EMBO J*, :4281-4290, 1992), extracellular-superoxide dismutase (Sandstrom, J., et al., *J Biol Chem*, **267**:18205-18209, 1992), and mast cell chymases (Matsumoto, R., et al., *J Biol Chem*, **270**:19524-19531, 1995), bind heparin via highly-basic binding regions which do not conform to any consensus sequence. In fact, in certain proteins, domains rich in basic amino acids have sometimes been shown to be unimportant for heparin binding. For example, the two heparin-binding consensus sequences identified in the FGFs were shown not to mediate heparin-binding (Wong, P., et al., *J Biol Chem*, **270**:25805-25811, 1995; Thompson, L. D., et al., *Biochem*, **33**:3831-3840, 1994). Therefore, there are likely other as yet undefined protein characteristics that must confer heparin-binding potential. Of relevance is the recent use of phage display technology to identify such novel heparin-binding sequences. This approach has generated three distinct HSPG-binding antibodies (van Kuppevelt, T. H., et al., *J Biol Chem*, **273** 21:12960-12966, 1998). Significantly, one of the sequences (GRRLKD, **SEQ. ID. NO:1**) contained a heparin-binding consensus sequence, while the others (SLRMNGCGAHQ, **SEQ. ID. NO:2**, and YYHYKVN, **SEQ. ID. NO:3**) did not. The latter two lack significant basic charge, and thus may bind HSPGs through non-ionic interactions. All three anti-HS antibodies showed specificity for heparin and HS but not

for other GAGs. Additionally, the antibodies all reacted differently towards HS from various sources, which would suggest a specificity in recognition of discrete HS molecules.

The paragraph starting on page 5, line 25 has been amended as follows:

Current approaches to design peptides which bind to heparin include Wakefield et al. (U.S. Patent No. 5,534,619, and U.S. Patent No. 5,919,761; Wakefield, T.W., et al., *J. Surg. Res.*, 56:586-593 1994; Wakefield, T.W., et al., *J. Surg. Res.*, 63:280-286, 1996) and Harris et al. (U.S. Patent No. 5,877,153). The Wakefield peptide sequences, specifically the grouping and spacing of the basic amino acids, are patterned after naturally-occurring protamines. The Harris et al. peptides are a series of single-chain and multi-chain peptides which incorporate arginines within a backbone of alanines. The spacings of the arginines are based on the heparin-binding sequence of antithrombin III. All the Harris peptides have AE as their N-terminal amino acid sequence.

The paragraph starting on page 9, line 19 has been amended as follows:

The peptides of the present invention are useful for counteracting the actions of heparin and other anticoagulant glycosaminoglycans on thrombin and Factor Xa activity, and may affect other proteins as well. Heparin is used routinely for anticoagulation. The interactions of exogenously administered heparin with the proteins of the coagulation and fibrinolytic pathways have been summarized in detail ([van Kuppevelt, T. H., et al., *J Biol Chem*, **273** (21):12960-12966, 1998]Conrad, H.E., Heparin-Binding Proteins, Academic Press, San Diego, 1998). These interactions are complex on many levels. The best-characterized targets for heparin are the procoagulant proteins thrombin and Factor Xa, which are inhibited by AT III when heparin binds to AT III. However, heparin acts at many sites. In some cases, the effect of heparin is anticoagulant and in other cases procoagulant. Some proteins, e.g. AT III, have heparin-binding consensus sites. However, the putative heparin-binding sequences are different for every known protein in these pathways, and the effects may depend on the 3-dimensional relationships of basic residues resulting from protein folding, rather than a short linear sequence, as is known

for the binding of heparin to AT III ([Lam, L. H., et al., *Biochem Biophys Res Commun*, **69**:570-577, 1976]Carrell, R.W., et al., *Structure* **2**:257-270, 1994). A tetrameric protein conformation of platelet factor 4 (PF4) is required for long-chain heparin binding ([Lee, M. K., and A. D. Lander, *Proc Nat Acad Sci USA*, **88**:2768-2772, 1991; Maccarana, M., et al., *J Biol Chem* **268**:23898-23905, 1993; Parthasarathy, N., et al., *J Biol Chem*, **269**:22391-22396, 1994]Rucinski, B., et al, *Thromb Hemostas* **63**:493-498, 1990; Ibel, K., et al, *Biochim Biophys Acta* **870**:58-63, 1986; Talpas, C.J., et al, *Biochim Biophys Acta* **1078**:208-218, 1991). Formation of a two-protein complex (PAI-1/vitronectin) involves the vitronectin heparin binding site ([Lindahl, U., et al., *J Biol Chem*, **273**:24979-24982, 1998; Fromm, J. R., et al., *Arch Biochem Biophys*, **343** (1):92-100, 1997]Kost, C.W. et al, *J Biol Chem* **267**:12098-12105, 1992; Deng, G., et al, *J Cell Biol* **134**:1563-1571, 1996) and therefore could be disrupted by heparin. The inactivated AT III/thrombin complex is released from the endothelial surface, binds as a complex to vitronectin, and then is taken up for catabolism by binding of the vitronectin heparin-binding domain to HSPG on the endothelium ([Robbins, S. L., and R. S. Cotran, *Pathological Basis of Disease*. W.B. Saunders, Philadelphia. 598-613 pp., 1979; Zarge, J. I., H. P., and H. P. Greisler. In *Principles of Tissue Engineering*. R. P. Lanza, R. Langer, and W. L. Chick, editors. Academic Press, Austin,. 349-364, 1997]Hogasen, J., et al, *J Biol Chem* 267:23076-23082, 1996; deBoer, H., et al, *J Biol Chem* 94:1279-1283, 1993).

The paragraph starting on page 10, line 16 has been amended as follows:

Heparin is a complex mixture of polysaccharides. Some of the interactions require long-chain heparins (AT III for inactivation of thrombin and binding to thrombin, HC II, PF4, and thrombospondin) while others depend on or can function with low molecular weight heparin chains(AT III for inhibition of Factor Xa, vitronectin, TFPI) ([van Kuppevelt, T. H., et al., *J Biol Chem*, **273** (21):12960-12966, 1998]Conrad, H.E., Heparin-Binding Proteins, Academic Press, San Diego, 1998). To further complicate the situation, specific sequences within the heparin chains may be required for interactions with the different proteins ([van Kuppevelt, T. H., et al., *J Biol Chem*, **273** (21):12960-12966, 1998]Conrad, H.E., Heparin-Binding Proteins, Academic Press, San Diego,

1998), and all naturally-occurring heparins and heparan sulfates are very diverse in their carbohydrate structures. The catabolism of the higher molecular weight heparins in the plasma results in a constantly changing spectrum of actual heparin chains that are available for reaction with the various proteins, and thus the nature of the possible anticoagulation or fibrinolytic reactions will change over the hours after the dosage is given. Finally, many other plasma proteins that are not involved in the coagulation or fibrinolytic processes can bind heparin, and variations in the concentration and nature of these proteins in different individuals can influence the availability of heparin for these two pathways. Thus specific single peptides or combinations of peptides may target specific interactions between heparins and cell surface or plasma proteins to get the greatest effectiveness and minimize adverse reactions.

The paragraph starting on page 11, line 3 has been amended as follows:

It is often necessary to reverse the effects of heparin when anticoagulation has reached a stage at which hemorrhage becomes a threat, notably after the routine use of heparin for anticoagulation during cardiopulmonary bypass, and in patients who develop an endogenous heparin-like coagulation inhibitor. The most commonly used anti-heparin drug is protamine, a mixture of basic proteins from fish sperm nuclei, that contains a high concentration of the amino acid arginine. When injected into a person who has been treated with heparin, it complexes rapidly to the heparin, thereby neutralizing its activity. Protamine also has numerous side effects including pulmonary hypotension that are difficult to control and provide significant health risks to the patient. Also, since Protamine is a poorly-defined and potentially variable product, dosage determination can be problematic. Importantly, Protamine has been shown to be ineffective for neutralization of low molecular weight heparins and the non-heparin glycosaminoglycan anticoagulant Orgaran. Well-defined heparin-or other GAG-binding peptides could be of considerable utility for reversing overdose of these specific anticoagulant preparations. Carson and co-workers ([Munro, M. S., et al., *Trans Am Soc Artif Intern Organs*, **27**:499-503, 1983]Lui, S., et al, *J Biol Chem* **94**:1739-1744, 1997) have identified a heparin-binding peptide from an epithelial/endothelial cell surface protein that has some ability to

neutralize heparin effects on thrombin generation, but optimal effects were found only at high peptide concentrations and low heparin and low thrombin concentrations. Preliminary data in the present invention suggest that the Cardin and serglycin peptides reverse the heparin effect on thrombin at several-fold lower peptide concentrations and 7-fold higher thrombin concentrations than the peptide described by Carson and co-workers. We have also shown that several of the peptides are effective neutralizers of low molecular weight heparin (Enoxaparin, Lovenox) and Orgaran in vitro, and of Lovenox in vivo in rats, in accordance with their affinity constants for low molecular weight heparin in vitro. Thus the peptides described in this application may have important clinical applications, especially if they can be targeted to specific reactions in the relevant pathway and to specific classes of heparins.

The paragraph starting on page 12, line 6 has been amended as follows:

Multiple interactions between the proteins of the coagulation and fibrinolysis pathways and endothelial cell surface PGs generate a complex surface on which ongoing coagulation and fibrinolysis are normally balanced to create a non-thrombotic state. The heparan sulfate PGs (HSPGs) of the endothelium mediate antithrombotic/anticoagulant function through binding and activation of Antithrombin III (AT III) and binding of tissue factor pathway inhibitor (TFPI). AT III bound to endothelium heparan sulfate can inactivate both thrombin and Factor Xa. TFPI binds to Factor Xa and this complex then interacts with the Factor VIIa/tissue factor complex to inactivate both Factors VIIa and Xa. Adherence of TFPI to the endothelium via the HSPG protects against proteolysis of the heparin-binding C-terminal domain ([Thompson, L. D., et al., *Biochem*, **33**:3831-3840, 1994]Nordfang, O., et al, *Biochem.* **30**:10371-10376, 1991); without this domain, activity is lost. Heparin-binding peptides such as those described in this study could behave similarly to platelet factor 4 (PF4) in that they could bind to the heparan sulfates on the endothelial surface. For example, docking of a peptide onto the heparan sulfate chain in a reversible manner could protect the GAG from degradation by platelet heparitinase released by aggregating platelets at the site of a developing thrombus, leaving the GAG able to resume its antithrombotic function in a shorter time frame than

would be required for resynthesis. On the other hand, a peptide with a very high affinity for the AT III-binding sequences of endothelial heparan sulfate could block the binding and therefore the activity of AT III and provide a more favorable surface for clot formation, thus promoting wound healing.

The paragraph starting on page 20, line 15 has been amended as follows:

FIG. 1 Calculation of heparin-binding affinities of peptides containing heparin-binding consensus sequences. Retardation coefficients (R) for the migration of low M_r ^{125}I-tyramine-heparin through peptides were determined from ACE gel electrophoretograms, and are plotted against peptide concentration as detailed in Materials and Methods. Smooth curves represent non-linear least-squares fits to the equation $R= R_\infty/\{1+ (K_d/[peptide])^n\}$. Peptides containing single consensus sequences (AKKARA, SEQ. ID. NO:4, -●- or ARKKAAKA, SEQ. ID. NO:5, -O-) do not bind heparin with a measurable affinity; in contrast, significant heparin-binding is seen with peptides containing multiple heparin-binding consensus sequences and increases as a function of peptide M_r.

$(AKKARA)_2$ (SEQ. ID. NO:14), -X-, $K_d\cong$ 40 μM; $(ARKKAAKA)_2$ (SEQ. ID. NO:6), -■-, $K_d\cong$ 6 μM; $(AKKARA)_3$ (SEQ. ID. NO:7),-□-, $K_d\cong$ 2 μM; $(ARKKAAKA)_3$ (SEQ. ID. NO:43), -▲-, $K_d\cong$ 135 nM and $(ARKKAAKA)_4$ (SEQ. ID. NO:8), -Δ-, $K_d\cong$ 40 nM.

The paragraph starting on page 20, line 27 has been amended as follows:

FIG. 2 Calculation of heparin-binding affinities of SG peptides. R values for the migration of low M_r ^{125}I-tyramine-heparin through peptides containing sequences native to the mouse (YPARRARYQWVRCKP, SEQ. ID. NO:9, -O -) or human (YPTQRARYQWVRCNP, SEQ. ID. NO:10, -●-) SG PG core proteins were determined from ACE gel electrophoretograms as detailed in Materials and Methods. SG peptides displayed relatively strong affinities for heparin ($K_d\cong$ 200 and 900 nM for the mouse and human peptides, respectively), in comparison to peptides of similar size which contain

multiple repeats of heparin-binding consensus sequences [e.g. $(AKKARA)_3$ (SEQ. ID. NO:7) $K_d \cong$ 2000 nM, $(ARKKAAKA)_2$ (SEQ. ID. NO:6) $K_d \cong$ 6000 nM; Table I]. Peptide AAARRARAAAARAKA (SEQ. ID. NO:11), (-■-) displayed negligible heparin-binding affinity ($K_d \cong$ 75 μM) indicating the importance of the non-basic residues to heparin-binding. YPARRARYQWVRCKP (SEQ. ID. NO:9)-heparin binding in the presence of β-ME (YPARRARYQWVRCKP, SEQ. ID. NO:9,+ β-ME, -X-), was decreased by over 20-fold ($K_d \cong$ 4 μM). Replacement of cysteine by alanine in the mouse SG peptide (YPARRARYQWVRAKP, SEQ. ID. NO:12, -□-) further reduced heparin-binding affinity ($K_d \cong$ 36 μM).

The paragraph starting on page 21, line 11 has been amended as follows:

FIG. 3 CD spectroscopy of $(AKKARA)_6$ (SEQ. ID. NO:13) in the presence or absence of low M_r heparin. CD spectra measurements of $(AKKARA)_6$ (SEQ. ID. NO:13) in the absence of heparin (1: 0,-●-), reveals peaks at 195 nm and 216 nm and a crossover at 210 nm, indicative of an extended charged coil conformation. Upon heparin addition (1: 0.25, -O-; 1: 0.50, -X-), the peptide conformation is altered and at a 1:1 peptide-heparin ratio (-■-) the peptide becomes α-helical with characteristic α-helical peaks at approximately 190, 207, and 222 nm. Excess heparin (1: 2, -□-, or 1: 4, -▲-) disrupts this interaction, and the spectra resembles that of a protein in a random coil conformation. Spectra are heparin and/or blank (water) corrected.

The paragraph starting on page 21, line 21 has been amended as follows:

FIG. 4 CD spectroscopy of $(AKKARA)_2$ (SEQ. ID. NO:14) in the presence or absence of low M_r heparin. The CD spectra measurements of $(AKKARA)_2$ (SEQ. ID. NO:14) in the absence of heparin (1: 0, -●-), indicates a charged coil conformation (peaks at 195 and 216 nm, crossover at 210 nm). However, in contrast to the heparin-induced conformational change seen for the high affinity heparin-binding peptide $(AKKARA)_6$ (SEQ. ID. NO:13), $(AKKARA)_2$ (SEQ. ID. NO:14), which binds heparin weakly, remains a charged coil in the presence of heparin (1: 0.25, -O-; 1: 0.50, -X-; 1: 0.75, -■-; 1: 1, -□-).

The paragraph starting on page 22, line 13 has been amended as follows:

FIG. 6 Affinity of $(ARKKAAKA)_4$ (SEQ. ID. NO:8) for HUVEC PGs and PG components. The peptide was analyzed for binding affinity to HUVEC PGs/GAGs by ACE, and the K_d of the peptide-PG/GAG interactions were calculated from binding plots as detailed in Experimental Procedures. Similar affinities (~300 nM) were obtained for total PGs, for PG samples devoid of HS GAGs via nitrous acid treatment (NA) or heparatinase I digestion (H), and for PGs devoid of CS GAGs via chondroitinase ABC (ABC) digestion. Liberation of GAG chains from the core protein by borohydride reduction (BH) of total PGs caused a 3-fold reduction in affinity ($K_d \cong$ 1200 nM).

The paragraph starting on page 23, line 14 has been amended as follows:

Peptides included in the present invention (all single letters represent conventional nomenclature designations for amino acids):

1. $(ARKKAAKA)_n$ (SEQ. ID. NO:5)
2. $(AKAAKKRA)_n$ (SEQ. ID. NO:15)
3. $(AKKARA)_n$ (SEQ. ID. NO:4)
4. $(ARAKKA)_n$ (SEQ. ID. NO:16)
4. YPARRARYQWVRCKP (SEQ. ID. NO:9)
5. YPTQRARYQWVRCNP (SEQ. ID. NO:10)

Examples of variations of the above peptide motifs included in this patent:

A. $(XBBBXXBX)_n$ or $(XBXXBBBX)_n$ where B denotes arginine (R), lysine (K) or a combination of the two, X denotes preferably but is not limited to alanine (A) or glycine (G), and $n \geq 2$. For example some possible permutations of the sequences covered by these patents will include but not be limited to:

$(ARRRAARA)_n$ **(SEQ. ID. NO:17)** $(ARRKAAKA)_n$ **(SEQ. ID. NO:18)**
$(AKKRAAKA)_n$ **(SEQ. ID. NO:19)** $(ARAARRRA)_n$ **(SEQ. ID. NO:20)**
$(ARAAKRKA)_n$ **(SEQ. ID. NO:21)**
$(GRRKGGRG)_n$ **(SEQ. ID. NO:22)** $(GRKKGGRG)_n$ **(SEQ. ID. NO:23)**
$(GKKKGGRG)_n$ **(SEQ. ID. NO:24)** $(GRGGKRRG)_n$ **(SEQ. ID. NO:25)**
$(GRGGKKRG)_n$ **(SEQ. ID. NO:26)**

B. $(XBBXBX)_n$ or $(XBXBBX)_n$ where B is arginine (R), lysine (K) or a combination of the two, X is preferably but not limited to alanine (A) or glycine (G), and $n \geq 2$. For example some possible permutations of the sequences covered by these patents will include but not be limited to:

$(ARRARA)_n$ **(SEQ. ID. NO:27)** $(ARKAKA)_n$ **(SEQ. ID. NO:28)**
$(ARARRA)_n$ **(SEQ. ID. NO:29)** $(ARAKKA)_n$ **(SEQ. ID. NO:30)**
$(GRRGKG)_n$ **(SEQ. ID. NO:31)** $(GKKGRG)_n$ **(SEQ. ID. NO:32)**
$(GRGRKG)_n$ **(SEQ. ID. NO:33)** $(GKGKRG)_n$ **(SEQ. ID. NO:34)**

C. Inclusion of a single cysteine (C) within 3 residues of either the N- or C-terminus as in YPARRARYQWVRCKP **(SEQ. ID. NO:9)**, or YPTQRARYQWVRCNP **(SEQ. ID. NO:10)** or in peptide sequences $(XBBBXXBX)_n$, $(XBXXBBBX)_n$, $(XBBXBX)_n$, or $(XBXBBX)_n$, where $n \geq 2$. For example some possible permutations of the sequences covered by these patents will include but not be limited to:

ARRKAARA-ARRKACRA **(SEQ. ID. NO:35)**
ARCAKKRA-ARAAKKRA-ARAAKKRA **(SEQ. ID. NO:36)**
ARRAKA-ARRAKA-ARRCKA **(SEQ. ID. NO:37)**
AKCKRA-AKAKRA **(SEQ. ID. NO:38)**

D. For any of the above peptides, this patent will also cover inclusion of the D- isomer forms of amino acids in place of the L-forms, or inclusions of any combinations of D- or

L-isomer forms to create reagents resistant to proteolytic degradation for in vitro and in vivo applications.

E. For any of the above peptides, this patent will also cover inclusion of any other amino acids in any X position.

F. This patent will also include peptides which incorporate multiple copies of the heparin-binding consensus sequences, but which are not necessarily arranged as concatamers, e.g., two such peptides may be ARKKAARAAAAAAAAARKKAARA **(SEQ. ID. NO:39)** or ARKKAARAAAAAAAAAAAAAAAARKKAARA **(SEQ. ID. NO:40)**

The paragraph starting on page 30, line 1 has been amended as follows:

Peptide-heparin interactions- To design small peptides which exhibit high affinities for heparin and for the GAG components of PGs, peptide sequences were modeled after proposed heparin-binding consensus sequence motifs. Thus, a collection of peptides containing one of two consensus sequence motifs, either XBBXBX or XBBBXXBX, as well as various modifications of these, were synthesized (Table I). As peptides containing a single heparin-binding sequence often show little or no affinity for heparin (Conrad, H. E, Heparin-Binding Proteins. Academic Press, San Diego, 1998), a strategy used here was to include consensus sequences in multiple copies within peptides. In initial studies we selected for synthesis the sequences $(AKKARA)_n$ **(SEQ. ID. NO:4)** or $(ARKKAAKA)_n$ **(SEQ. ID. NO:5)**, where n = 1-6. Alanine was included in the hydropathic positions because of its stabilizing activity on α-helices (Ferran, D. S., et al., *Biochem*, **31**:5010-5016, 1992) and the basic amino acids were chosen to represent those with the highest probability of occurrence in each basic position in the heparin-binding consensus sequences of native heparin-binding proteins (Cardin, A. D. and H. J. R. Weintraub, *Arteriosclerosis*, **9**:21-32, 1989). When single copies of either sequence were tested for heparin-binding by ACE, no affinities were detected. In contrast, peptides containing two copies of the consensus sequence exhibited weak but detectable affinities for heparin (< 6 μM), and peptides of higher molecular weight containing 4-6 copies of a

consensus sequence showed a marked increase in heparin-binding affinity (40-150 nM) (Fig. 1). The heparin-binding affinity of both the 6-mer and 8-mer tandem-repeat peptides reached a plateau as peptide length approached 30 amino acids [$(AKKARA)_5$ **(SEQ. ID. NO:41)**, $K_d \cong$ 90 nM and $(ARKKAAKA)_4$ **(SEQ. ID. NO:8)**, $K_d \cong$ 40 nM]. Larger peptides [$(AKKARA)_6$ **(SEQ. ID. NO:13)** and $(ARKKAAKA)_5$ **(SEQ. ID. NO:42)**] displayed similar affinities ($K_d \cong$ 100 and 50 nM respectively, Table I).

The paragraph starting on page 30, line 24 has been amended as follows:

To define the sequence and conformational features of the tandem-repeat peptides which confer their high affinity heparin-binding characteristics, peptides containing variants of one of the consensus sequences first tested, $(ARKKAAKA)_3$ **(SEQ. ID. NO:43)**, were synthesized. These included those in which alanines were replaced by other hydropathic residues, the spacings between consensus sequences were altered by removal or addition of alanine residues, or the potential of the peptides to form stable α-helices was inhibited by including proline residues at various positions. It was found that peptide affinity for heparin was decreased when alanine was replaced by glycine in all the hydropathic positions [$(ARKKAAKA)_3$ **(SEQ. ID. NO:43)** $K_d \cong$ 135 nM, $(GRKKGGKG)_3$ **(SEQ. ID. NO:44)** $K_d \cong$ 200 nM, $p < 0.01$]; less conservative substitutions had varying effects on heparin-binding affinity, i.e. for $(LRKKLGKR)_3$ **(SEQ. ID. NO:45)**, $K_d \cong$ 105 nM, affinity was unaffected; for $(TRKKLGKI)_3$ **(SEQ. ID. NO:46)**, $K_d \cong$ 740 nM, ($p < 0.01$) affinity was decreased (Table I).

The paragraph starting on page 31, line 4 has been amended as follows:

Two peptides were synthesized in which the spacings between adjacent consensus sequences were altered. Both increasing (ARKKAAKA-AAAA-ARKKAAKA-AAAA-ARKKAAKA, **SEQ. ID. NO:47**) or decreasing (ARKKAAKA-RKKAAKA-RKKAAKA, **SEQ. ID. NO:48**) the distance between consensus sequences resulted in decreased heparin-binding affinity ($K_d \cong$ 250 and 450 nM respectively). Inclusion of prolines also decreased the heparin-binding affinity, the degree of which was influenced by their position and number. Thus, the heparin-binding affinity decreased to 360 nM

when prolines were present in each tandem repeat in place of an alanine: $(ARKKPAKA)_3$ (SEQ. ID. NO:49); however, a weaker affinity was obtained when a single proline was substituted in the center of a series of three heparin-binding consensus sequences (ARKKAAKA-ARKKPAKA-ARKKAAKA (SEQ. ID. NO:50), $K_d \cong$ 730 nM, Table I).

The paragraph starting on page 31, line 15 has been amended as follows:

Other peptides synthesized and studied include sequences native to the mouse (YPARRARYQWVRCKP, SEQ. ID. NO:9) or human (YPTQRARYQWVRCNP, SEQ. ID. NO:10) serglycin (SG) core proteins, which contain either a single or partial consensus sequence, respectively. These showed significant affinities for heparin ($K_d \cong$ 200-900 nM, Table I and Fig. 2), despite their small sizes (about 2000 Da). To elucidate the basis for the strong heparin-binding features of these peptides, the ability of the basic residues to sustain high affinity binding was tested by studying a peptide which contained all the basic residues of the mouse sequence in their native positions, but in which all other residues were changed to alanines (AAARRARAAAARAKA, SEQ. ID. NO:11). A 350-fold decrease in heparin-binding affinity ($K_d \cong$ 72 μM) for this peptide indicated that the number and arrangement of basic residues in the mouse sequence was not sufficient for high affinity binding, and suggests the importance of one or more of the other non-basic residues (Fig 2). We next tested whether the C-terminal cysteine in the mouse SG peptide may promote peptide dimer formation, thereby influencing heparin-binding affinity. Thus, heparin-binding was tested by ACE under reducing conditions, and it was found that this treatment yielded negligible heparin-binding. Likewise, when the cysteine residue was replaced by an alanine in the native mouse SG sequence, (YPARRARYQWVRAKP, SEQ. ID. NO:12), heparin-binding affinity was again negligible; both results are consistent with the potential cross-linking function of the cysteine residues (Fig. 2).

The paragraph starting on page 32, line 4 has been amended as follows:

CD- The intrinsic structural properties of the peptides were explored using CD spectroscopy. Short peptides of known heparin-binding proteins containing heparin-

binding consensus sequences have previously been shown to fold into α-helical conformations. In doing so, the basic amino acids locate to one face of the helix, and thus are potentially exposed for binding. Peptides which displayed weak $(AKKARA)_2$ **(SEQ. ID. NO:14)**, moderate $(AKKARA)_4$ **(SEQ. ID. NO:51)**, and strong $(AKKARA)_5$ **(SEQ. ID. NO:41)** and $(AKKARA)_6$ **(SEQ. ID. NO:13)**, heparin-binding affinities, were analyzed by CD to characterize their degree of α-helical contents and propensities to form an α-helix. All peptides exhibit very similar spectra with peaks at 195 nm and 216 nm and a crossover at 210 nm [for example, see Fig. 3, $(AKKARA)_6$ **(SEQ. ID. NO:13)**, 1:0, -●-, and Fig. 4 $(AKKARA)_2$ **(SEQ. ID. NO:14)**, 1:0, -●-]. These spectra are indicative of an extended charged coil conformation that was previously reported for charged poly(L)-lysines and poly(L)-arginines (Gelman, R. A., et al., *Biopolymers*, **12**:541-558, 1973).

The paragraph starting on page 32, line 16 has been amended as follows:

Intrinsic CD of the peptides shows that they do not adopt α-helical conformations. To explore the conformational repertoire of the peptides and to record CD spectra for the α-helical conformations, peptides were analyzed by CD in the presence of the non-polar solvent TFE. Non-polar solvents are known to increase the degree of α-helicity of a peptide in solution by enhancing hydrogen bonding and electrostatic interactions (Adler, A. J., and G. D. Fasman, *J Phys Chem*, **75**:1516-1526, 1971). CD of $(AKKARA)_6$ **(SEQ. ID. NO:13)**, at 0.1 mg/ml containing 0, 10, 20, 30, 40, and 50% TFE (v/v) was measured. At TFE concentrations >30%, with an apparent maximal effect induced at 40% TFE, the peptide assumes an α-helical conformation with classic α-helical peaks at 206 and 220 nm and a cross over at 197 nm (data not shown).

The paragraph starting on page 32, line 26 has been amended as follows:

The CD spectra of $(AKKARA)_6$ **(SEQ. ID. NO:13)** recorded in the presence of increasing amounts of heparin (Fig. 3) demonstrates that a change from a charged coil conformation displayed in the absence of heparin (1: 0) occurs upon heparin addition (1: 0.25, 1: 0.50, 1: 1). Heparin induces a similar α-helical conformation at a 1: 1 peptide:

heparin ratio that was obtained in the presence of >30% TFE , with classic α-helical peaks at 190, 207, and 222 nm. At higher heparin concentrations (1: 2 or 1: 4) the α-helical form is lost and the spectrum resembles that of a random coil structure. This ability of excess GAG to disrupt the α-helical conformation of a polypeptide in solution has been reported previously (Gelman, R. A., et al., *Biopolymers*, **12**:541-558, 1973).

The paragraph starting on page 33, line 4 has been amended as follows:

This same heparin effect is not obtained for the weak heparin-binding peptide $(AKKARA)_2$ **(SEQ. ID. NO:14)** (Fig. 4). In the absence of heparin (1: 0), the peptide assumes a similar charged coil conformation as that observed for $(AKKARA)_6$ **(SEQ. ID. NO:13)**, but fails to display α-helical character in the presence of heparin (1: 0.25, 1: 0.50, 1: 0.075, or 1:1).

The paragraph starting on page 33, line 9 has been amended as follows:

Peptide-PG interactions- The interactions between consensus sequence peptides and PGs were also examined. For these experiments total PGs were isolated from HUVEC cultures, since HUVEC have been shown to express a variety of types of HS and CS PGs, including, for example, syndecans, perlecan, glypican and biglycan (Mertens, G., et al., *J Bio Chem*, **267** (28):20435-20443, 1992; Jarvelainen, H. T., et al., *J Biol Chem*, **266** (34):23274-23281, 1991). Thus, cell layer-associated and secreted ^{35}S-SO_4-radiolabeled PGs were purified by extraction with urea, and those PGs retained on DEAE after a 0.1 M NaCl rinse were studied for their binding to $(ARKKAAKA)_4$ **(SEQ. ID. NO:8)** by ACE (Fig. 5A, EC PGs). This peptide exhibited significant affinity for secreted HUVEC PGs, although the average affinity was somewhat weaker than that exhibited by the peptide for heparin (PG $K_d \cong$ 300 nM, heparin $K_d \cong$ 50 nM). Similar affinities were obtained for cell layer associated PGs (data not shown). Inspection of ACE gels in which secreted PGs were fractionated through peptides demonstrated the presence of at least two populations of PG evident as two distinct bands of radiolabeled material migrating through the peptide lanes with different mobilities (Fig. 5A, EC PGs). This difference in migration rate could indicate heterogeneity of the PG in size or charge. In contrast to the

heterogeneity seen in Fig. 5A, Fig. 5B shows that heparin migrates as a single band of radiolabeled material.

The paragraph starting on page 33, line 27 has been amended as follows:

Thus, to ascertain which GAG chains, as well as which PG component, (i.e. core protein, GAG chains, or both), were responsible for peptide-binding, total HUVEC PGs were subjected to various chemical and enzymatic degradations. Samples were then tested for their ability to bind to $(ARKKAAKA)_4$ **(SEQ. ID. NO:8)** PGs in which HS GAGs were chemically degraded by nitrous acid or enzymatically degraded by heparatinase I, were able to maintain comparable affinity for the peptide as was displayed by the total PG sample (Fig 5A, EC PGs/NA and Fig. 6). PGs in which CS GAG chains were digested with chondroitinase ABC were also able to maintain comparable affinity for the peptide. Release of GAG chains from cores by borohydride reduction resulted in a 3-4 fold diminished affinity (Fig. 6).

The paragraph starting on page 34, line 31 has been amended as follows:

In our initial experiments, families of peptides were synthesized that contained single or multiple copies of heparin-binding consensus sequences. When their heparin-binding was examined by ACE, peptides containing single sequences showed no measurable affinity for heparin. This result is as expected since peptides carrying single heparin-binding sequences found in native proteins often fail to display significant heparin-binding (Conrad, H. E., Heparin-Binding Proteins. Academic Press, San Diego, 1998), but they may contain multiple consensus sequences that come into proximity upon protein folding or multimerization, thereby enhancing heparin-binding through cooperativity (Huntington, J. A., et al., *Biochem*, **35**:8495-8503, 1996). In contrast, the affinity of peptides $(AKKARA)_n$ **(SEQ. ID. NO:4)** or $(ARKKAAKA)_n$ **(SEQ. ID. NO:5)** ranged from weak ($K_d \cong$ 6-40 μM) when n= 2, to strong ($K_d \cong$ 50-100 nM) when n= 3-6. These latter affinities are in the range of those displayed by heparin-binding proteins such as bFGF ($K_d \cong$ 10 nM), or type I collagen, ($K_d \cong$ 100-200 nM) (San Antonio, J. D., et al., *Biochem*, **32**:4746-47, 1993). However, the fact that the peptides are roughly 4 times

smaller than bFGF and 100 times smaller than type I collagen highlights their significant heparin-binding abilities. The affinity appeared to plateau at $n \geq 5$, or 36-40 amino acids, suggesting that peptides of approximately 30-32 amino acids were of sufficient length to occupy all available binding sites on low M_r heparin, and that additional amino acid residues beyond this did not contribute to heparin binding due to a lack of available ligand. However, this hypothesis can not be tested without knowing the M_r distribution of the heparin used in these experiments.

The paragraph starting on page 35, line 20 has been amended as follows:

Other experiments examined heparin binding by peptides including sequences native to proteins which contain a single or partial heparin-binding consensus sequence. Results again suggested the critical nature of peptide M_r and number of consensus sequences to heparin-binding. Thus, surprisingly, a strong heparin-binding affinity was displayed by a peptide corresponding to the mouse SG proteoglycan core protein containing a single consensus sequence, YPARRARYQWVRCKP **(SEQ. ID. NO:9)** ($K_d \cong$ 200 nM). However, the affinity was diminished over 200-fold by disulfide reduction, or replacement of the cysteine with alanine, thus implying that its strong heparin-binding relies on peptide dimerization, and that the other residues flanking the consensus sequence were of little consequence. Indeed, others have shown that inclusion of cysteines near peptide termini to promote disulfide bond formation may improve peptide-ligand binding (Starovasnik, M. A., et al., *Proc Natl Acad Sci USA*, **94**:10080-10085, 1997); our results suggest this to be a simple strategy to greatly enhance the affinity of peptides for heparin. SG, cerebroglycan (with PRRLRL, **SEQ. ID. NO:52)** (Stipp, C. S., et al., *J Cell Biol*, **124**:149-160, 1994), and perlecan (with TRRFRD, **SEQ. ID. NO:53)** (Murdoch, A. D., et al., *J Biol Chem*, **267** (12):8544-8557, 1992) are among the few PGs that contain heparin-binding consensus sequences on their core proteins. Interestingly, the SG core protein, which carries many heparin chains, migrates at twice its predicted molecular weight on PAGE gels under reducing conditions (Perin, J.-P., et al., *Biochem J*, **255**:1007-1013, 1988); suggesting dimerization. This could result from GAG chains of one PG binding to the core protein of another, or core-core associations

through disulfide bonding. The potential physiological function of such PG-PG interactions remains to be explored.

The paragraph starting on page 36, line 25 has been amended as follows:

Molecular modeling of consensus sequences in native heparin-binding proteins predicts their presence within α-helical regions (Cardin, A. D. and H. J. R. Weintraub, *Arteriosclerosis*, **9**:21-32, 1989). Additionally, GAG-directed conformational changes on polypeptides such as poly(L)-lysine and poly(L)-arginine have been identified (Gelman, R. A., et al., *Biopolymers*, **12**:541-558, 1973; Gelman, R. A., and J. Blackwell, *Arch Biochem Biophys*, **159**:427-433, 1973; Gelman, R. A., and J. Blackwell, *Biopolymers*, **13**:139-156, 1974). Aqueous solutions of these polypeptides at neutral pH were shown by CD to adopt charged coil conformations, and to display α-helical conformations in the presence of heparin. Our results showed that peptides of the type $(AKKARA)_n$ **(SEQ. ID. NO:4)** have charged coil conformations at neutral pH. In the presence of heparin, however, a peptide that showed high affinity for heparin, $(AKKARA)_6$ **(SEQ. ID. NO:13)**, underwent a conformational change to an α-helix. In the presence of excess heparin, a further conformational change produced a random coil structure. In contrast, a peptide which displayed weak heparin-binding, $(AKKARA)_2$ **(SEQ. ID. NO:14)**, failed to undergo any conformational change. Thus, the solution conformation of a peptide and its propensity to change conformation in the presence of heparin may be an indication of its ability to bind to heparin strongly. These data and those from experiments examining the effects of including prolines in peptides, which are known to disrupt the α-helical conformation, suggest that peptide secondary structure facilitates heparin-binding.

The paragraph starting on page 37, line 12 has been amended as follows:

Here we also examined the interaction between the high affinity heparin-binding peptide $(ARKKAAKA)_4$ **(SEQ. ID. NO:8)** and EC PGs. Results showed that ECs secreted several types of PGs/GAGs which displayed significant affinities for $(ARKKAAKA)_4$ **(SEQ. ID. NO:8)** ($K_d \cong 300$ nM). ACE gel images revealed the resolution of multiple PG/GAG species after their migration through the peptide-

containing lanes, suggesting heterogeneity in PG/GAG charge, size, and/or binding affinities. It was found that the CSPGs or HSPGs likely bind the peptide similarly, since affinity was maintained even after treatment of total PGs with nitrous acid, which selectively degrades HS GAGs, heparatinase I or chondroitinase ABC. The free GAG chains had 3-4 fold lower affinity than the intact PGs. Thus the core proteins of certain EC PGs either may contribute to binding directly, or act as a tether to bring multiple GAGs into proximity for cooperative binding. Similar observations have been made previously for cartilage PG-type II collagen interactions (Toole, B., *J Biol Chem* **251**:895-897, 1976) and SG-type I collagen interactions (Schick, B. P., et al., *J Cell Physiol*, **172**:87-93, 1997). Our results are inconsistent with carbohydrate sequence selectivity in the binding of these peptides with EC PGs, since similar affinities for peptides were displayed by either total EC PGs or its CSPG fraction.

The paragraph starting on page 42, line 7 has been amended as follows:

The maximal concentration of Lovenox in the plasma was 0.5-1.0 U/ml anti-Factor Xa activity for all the animals tested. The maximal plasma heparin concentration was found by 2-2.5 minutes after injection. About half the Lovenox was cleared from the circulation by 25-30 minutes after injection, in an approximately linear fashion for 15 minutes and more slowly thereafter. Panel A shows a representative clearance curve. The three peptides at dosages shown in panels B-D caused no removal of Lovenox above that due to direct clearance from the circulation alone (Panel A). The peptide $(ARKKPAKA)_3$ **(SEQ. ID. NO:49)** (Panel D) appeared to delay clearance of the heparin from the circulation.

The paragraph starting on page 42, line 16 has been amended as follows:

Protamine and three of our high affinity heparin-binding peptides [$(AKKARA)_6$ **(SEQ. ID. NO:13)**, $(ARKKAAKA)_5$ **(SEQ. ID. NO:42)**, and $(ARKKAAKA)_4$ **(SEQ. ID. NO:8)**] neutralized the Lovenox concentration by at least 50%-80% within 2 minutes of injection of the peptide, the less tightly-binding mouse serglycin Cardin-site peptide

was somewhat less effective, and there was no further clearance of Lovenox in all cases for the remainder of the 30-minute experiment (Panels E-J).

Table I starting on page 43, line 2 has been amended as follows:

TABLE I

Heparin-binding affinity of peptides containing heparin-binding consensus sequences

Peptides were analyzed for heparin-binding affinity by ACE, and K_{ds} of peptide-heparin interactions were calculated from binding plots as detailed in Experimental Procedures. Each sample was tested for heparin-binding three to eleven times, with an average of four times. K_d represents an average of data obtained for all trials ± standard deviation (S.D.); $^{*}p$ values < 0.01 versus $(AKKARA)_4$ (SEQ. ID. NO:51) and $^{**}p$ values < 0.01 versus $(AKKARA)_3$ (SEQ. ID. NO:7); $^{*}p$ values < 0.01 and $^{**}p$ values < 0.05 versus $(ARKKAAKA)_3$ (SEQ. ID. NO:43).

Peptide Sequence	M_r	K_d, (nM) ± S.D.
XBBXBX tandem repeats		
AKKARA (SEQ. ID. NO:4)	644	Not detectable
$(AKKARA)_2$ (SEQ. ID. NO:14)	1270	40,000 ± 18,000
$(AKKARA)_3$ (SEQ. ID. NO:7)	1895	1,900 ± 210*
$(AKKARA)_4$ (SEQ. ID. NO:51)	2520	174 ± 19
$(AKKARA)_5$ (SEQ. ID. NO:41)	3146	94 ± 41*
$(AKKARA)_6$ (SEQ. ID. NO:13)	3770	104 ± 32*
$(ARRAKA)_3$ (SEQ. ID. NO:54)	1979	900 ± 170**
XBBBXXBX tandem repeats		
ARKKAAKA (SEQ. ID. NO:5)	843	Not detectable
$(ARKKAAKA)_2$ (SEQ. ID. NO:6)	1668	6,200 ± 3,000
$(ARKKAAKA)_3$ (SEQ. ID. NO:43)	2493	135 ± 54
$(ARKKAAKA)_4$ (SEQ. ID. NO:8)	3318	42 ± 15*
$(ARKKAAKA)_5$ (SEQ. ID. NO:42)	4143	51 ± 11*
$(ARRRAARA)_3$ (SEQ. ID. NO:55)	2745	72 ± 22**
$(AKAAKKRA)_3$ (SEQ. ID. NO:56)	2493	132 ± 93
XBBBXXBX tandem repeats with hydropathic position modifications		
$(AKRKKAAKA)_3$ (SEQ. ID. NO:57)	2878	75 ± 41
$(GRKKGGKG)_3$ (SEQ. ID. NO:44)	2325	200 ± 98*
$(LRKKLGKR)_3$ (SEQ. ID. NO:45)	2959	105 ± 37
$(TRKKLGKI)_3$ (SEQ. ID. NO:46)	2794	737 ± 350*
$(ARKKPAKA)_3$ (SEQ. ID. NO:49)	2571	360 ± 127*
ARKKAAKAARKKPAKAARKKAAKA (SEQ. ID. NO:50)	2519	730 ± 340*
ARKKAAKARKKAKARKKAAKA (SEQ. ID. NO:58)	2351	450 ± 95*
ARKKAAKAAAAAARKKAAKAAAAAARKKAAKA (SEQ. ID. NO:47)	3062	254 ± 137*
Native or modified serglycin sequences		
YPARRARYQWVRCKP (SEQ. ID. NO:9)	1948	187 ± 54
YPTQRARYQWVRCNP (SEQ. ID. NO:10)	1936	817 ± 170
YPARRARYQWVRAKP (SEQ. ID. NO:12)	1918	37,000± 6,700

AAARRARAAAARAKA **(SEQ. ID. NO:11)**	1482	72,000±60,000

Table II starting on page 45, line 1 has been amended as follows:

TABLE II

Percent Reversal of anti-Factor Xa Activity by Peptides in Vitro

Lovenox (Rhone Poulenc Rohrer), Orgaran (Organon), and unfractionated heparin (Sigma Chemical Co.) were added to plasma to obtain 0.5 U/ml anti-Factor Xa activity as determined by the Stachrom Heparin assay. The ATIII/heparin complex was allowed to form, the peptide was added at the indicated concentrations, and the residual Factor Xa activity was measured by a chromogenic assay according to the directions supplied with the kit. The per cent reversal of heparin-like activity was determined. Each number represents the average of one-three duplicate or triplicate determinations, in which replicates were ± 2%. Data were not obtained for areas left blank in the Table.

		Peptide concentration in plasma		
Peptide	Type of Heparin	0.6 mg/ml	0.4mg/ml	0.12 mg/ml
$(AKKARA)_2$ **(SEQ. ID. NO:14)**	Lovenox	61.5%		
	Orgaran	37.0%		
	Heparin	67.0%		
$(AKKARA)_4$ **(SEQ. ID. NO:51)**	Lovenox	73%		
	Orgaran	75%		
	Heparin	99%		
$(AKKARA)_6$ **(SEQ. ID. NO:13)**	Lovenox	82.5%	86.5%	86%
	Orgaran	77%	81.5%	74.5%
	Heparin	93%	97.5%	99%
$(ARKKAAKA)_4$ **(SEQ. ID. NO:8)**	Lovenox	73%		
	Orgaran	70.5%		
	Heparin	87.5%		
$(ARKKAAKA)_5$ **(SEQ. ID. NO:42)**	Lovenox	81.5%	71.0%	68.5%
	Orgaran	83.0%	75.0%	75.0%
	Heparin	94.5%	93.0%	64.5%
$(AKAAKKRA)_3$ **(SEQ. ID. NO:56)**	Lovenox	93.5%		
	Orgaran	72.0%		
	Heparin	94.0%		

$(ARKKPAKA)_3$ (SEQ. ID. NO:49)	Lovenox	100%	62.5%	62.0%
	Orgaran	66.5%	67.5%	45.0%
	Heparin	99.0%	50.0%	90.0%

$(ARRRAARA)_3$ (SEQ. ID. NO:55)	Lovenox	67.5%
	Orgaran	67.0%
	Heparin	57.5%

ARKKAAKAARKKPAKAARKKAAKA (SEQ. ID. NO:50)

Lovenox	83.0%
Orgaran	67.0%
Heparin	57.5%

YPARRARYQWVRCKP (SEQ. ID. NO:9) (murine serglycin)

Lovenox	25.0%
Orgaran	16.5%
Heparin	77.0%

YPARRARYQWVRAKP (SEQ. ID. NO:12) (murine serglycin, cysteine replaced with alanine)

Lovenox	0%
Orgaran	2.0%
Heparin	25.0%

YPTQRARYQWVRCNP (SEQ. ID. NO:10) (human serglycin)

Lovenox	0%
Orgaran	5%
Heparin	90%

Table III starting on page 47, line 1 has been amended as follows:

TABLE III

Reversal of Anti-Thrombin Effects of Unfractionated Heparin by Peptides in Vitro

Plasma was obtained from normal donors. Thrombin concentration was standardized to produce a clotting time of 20-22 seconds. Heparin was added at 0.5U/ml. The clotting time for heparin alone was approximately 3 minutes. One minute after addition of heparin to the plasma, the peptides were added in concentrations ranging from 1-200 ug/ml. After one minute, thrombin was added and the clotting time determined. The clotting time was normalized at the peptide concentrations shown below.

Peptide	μg peptide/ml heparinized plasma needed to normalize thrombin time
$(AKKARA)_6$ **(SEQ. ID. NO:13)**	5
$(AKKARA)_4$ **(SEQ. ID. NO:51)**	10
$(AKKARA)_2$ **(SEQ. ID. NO:14)**	> 50
$(ARKKAAKA)_5$ **(SEQ. ID. NO:42)**	21
$(AKAAKKRA)_3$ **(SEQ. ID. NO:56)**	>50
YPTQRARYQWVRCNP **(SEQ. ID. NO:10)** (human serglycin)	100
YPARRARYQWVRCKP **(SEQ. ID. NO:9)** (mouse serglycin)	15

In the Claims:

Claims 64 and 65 have been amended as follows:

64. (Once Amended) The synthetic murine serglycin peptide having the sequence YPARRARYQWVRCKP **(SEQ. ID. NO:9)**.

65. (Once Amended) The synthetic human serglycin peptide having the sequence YPTQRARYQWVRCNP **(SEQ. ID. NO:10)**.